

AMENDMENTS TO THE CLAIMS

Claim 1 (Currently Amended): A method for producing a protein having an antithrombotic activity, which comprises replacing, in a protein that has an amino acid sequence having a ~~homology~~ a sequence identity of not less than 30% to the amino acid sequence of SEQ ID NO: 1 and forms a ~~secondary or tertiary structure~~, from N-terminus to C-terminus, composed of a first  $\beta$  strand ( $\beta 1$ ), a first  $\alpha$  helix ( $\alpha 1$ ), a second  $\alpha$  helix ( $\alpha 2$ ), a second  $\beta$  strand ( $\beta 2$ ), a loop, a third  $\beta$  strand ( $\beta 3$ ), a fourth  $\beta$  strand ( $\beta 4$ ) and a fifth  $\beta$  strand ( $\beta 5$ ) in this order from the amino terminus, at least one amino acid residue in a region from  $\alpha 2$  to  $\beta 2$ , a region from  $\beta 3$  to  $\beta 4$ , or in the regions from  $\alpha 2$  to  $\beta 2$  and from  $\beta 3$  to  $\beta 4$  so that electric charge of the amino acid residue is substituted towards positive direction as compared to the unsubstituted amino acid.

Claim 2 (Previously Presented): The method according to Claim 1, wherein at least one acidic amino acid residue in the region from  $\alpha 2$  to  $\beta 2$ , a region from  $\beta 3$  to  $\beta 4$ , or in the regions from  $\alpha 2$  to  $\beta 2$  and from  $\beta 3$  to  $\beta 4$  is replaced with a neutral amino acid residue to change electric charge of the amino acid residue towards positive direction as compared to the unsubstituted amino acid.

Claim 3 (Previously Presented): The method according to Claim 1, wherein the protein originates from *Crotalus horridus horridus*.

Claim 4 (Currently Amended): The method according to Claim 1, wherein the region from  $\alpha 2$  to  $\beta 2$  in the protein corresponds to the sequence of the amino acid ~~numbers~~ residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1 and the region from  $\beta 3$  to  $\beta 4$  corresponds to the sequence of the amino acid ~~numbers~~ residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1.

Claim 5 (Currently Amended): The method according to Claim 4, wherein at least one acidic amino acid residue of which  $\alpha$  carbon atom exists within 10 Å from the  $\alpha$  carbon atom of the arginine residue of the amino acid ~~number~~ residue 103 in the amino acid sequence of SEQ ID NO: 1 is replaced with a neutral amino acid residue.

Claim 6 (Currently Amended): The method according to Claim 5, wherein the acidic amino acid residue is at least one residue selected from the aspartic acid residue of the amino acid ~~number~~ residue 54, the aspartic acid residue of the amino acid ~~number~~ residue 101 and the glutamic acid residue of the amino acid ~~number~~ residue 106 in the amino acid sequence of SEQ ID NO: 1.

Claim 7 (Previously Presented): The method according to Claim 1, which further comprises deleting a region containing the loop structure existing between  $\beta 2$  and  $\beta 3$  in such a manner that the secondary or tertiary structures of  $\beta 2$  and  $\beta 3$  are maintained, or replacing the region with one or more amino acid residue(s) in a number required to maintain the secondary or tertiary structures of  $\beta 2$  and  $\beta 3$ , said amino acid residue(s) being selected from the group consisting of a glycine residue, an alanine residue, a serine residue and a cysteine residue.

Claim 8 (Original): The method according to Claim 7, wherein the region containing the loop structure existing between  $\beta 2$  and  $\beta 3$  is replaced with an amino acid sequence composed of four glycine residues.

Claim 9 (Currently Amended): The method according to Claim 1, which further comprises covalently bonding a polyoxyalkylpolyol group to the protein.

Claim 10 (Currently Amended): The method according to Claim 9, wherein the protein contains a cysteine residue corresponding to a cysteine residue of the amino acid

~~number~~ residue 81 in the amino acid sequence of SEQ ID NO: 1, and the polyoxyalkylpolyol group is bonded to said cysteine residue.

Claim 11 (Previously Presented): The method according to Claim 9, wherein the polyoxyalkylpolyol group is a polyethylene glycol group.

Claims 12. – 20. (Canceled).

Claim 21 (Withdrawn): A DNA coding for a protein comprising an amino acid sequence with a homology of not less than 30% to the amino acid sequence of SEQ ID NO: 1 and forms a secondary or tertiary structure composed of a first  $\beta$  strand ( $\beta$ 1), a first  $\alpha$  helix ( $\alpha$ 1), a second  $\alpha$  helix ( $\alpha$ 2), a second  $\beta$  strand ( $\beta$ 2), a loop, a third  $\beta$  strand ( $\beta$ 3), a fourth  $\beta$  strand ( $\beta$ 4) and a fifth  $\beta$  strand ( $\beta$ 5) in this order from the amino terminus, and wherein at least one amino acid residue in a region from  $\alpha$ 2 to  $\beta$ 2, a region from  $\beta$ 3 to  $\beta$ 4, or in the regions from  $\alpha$ 2 to  $\beta$ 2 and from  $\beta$ 3 to  $\beta$ 4 is replaced so that electric charge of the amino acid residue is substituted towards positive direction as compared to the unsubstituted amino acid, said protein being the following (a) or (b):

(a) a protein, in which the region from  $\alpha$ 2 to  $\beta$ 2 has the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1 and the region from  $\beta$ 3 to  $\beta$ 4 has the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1;

(b) the protein according to (a), in which substitution, insertion or deletion of one amino acid residue is included in the region from  $\alpha$ 2 to  $\beta$ 2 having the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1, or the region from  $\beta$ 3 to  $\beta$ 4 having the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1; and

wherein said protein has antithrombotic activity.

Claim 22 (Withdrawn): A method for producing a protein having antithrombotic activity, which comprises:

culturing a host microorganism transformed with the DNA as claimed in Claim 21;  
and collecting the protein from a culture.

Claim 23 (Withdrawn): The method for producing a protein having antithrombotic activity according to Claim 22, further comprising:

bonding a polyoxyalkylpolyol group to the collected protein.

Claim 24 (Canceled):

Claim 25 (Withdrawn): A DNA coding for a protein comprising an amino acid sequence with a homology of not less than 30% to the amino acid sequence of SEQ ID NO: 1 and forms a secondary or tertiary structure composed of a first  $\beta$  strand ( $\beta 1$ ), a first  $\alpha$  helix ( $\alpha 1$ ), a second  $\alpha$  helix ( $\alpha 2$ ), a second  $\beta$  strand ( $\beta 2$ ), a loop, a third  $\beta$  strand ( $\beta 3$ ), a fourth  $\beta$  strand ( $\beta 4$ ) and a fifth  $\beta$  strand ( $\beta 5$ ) in this order from the amino terminus, and wherein at least one amino acid residue in a region from  $\alpha 2$  to  $\beta 2$ , a region from  $\beta 3$  to  $\beta 4$ , or in the regions from  $\alpha 2$  to  $\beta 2$  and from  $\beta 3$  to  $\beta 4$  is replaced so that electric charge of the amino acid residue is substituted towards positive direction as compared to the unsubstituted amino acid, said protein being the following (a) or (b):

(a) a protein, in which the region from  $\alpha 2$  to  $\beta 2$  has the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1 and the region from  $\beta 3$  to  $\beta 4$  has the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1;

(b) the protein according to (a), in which substitution, insertion or deletion of one amino acid residue is included in the region from  $\alpha 2$  to  $\beta 2$  having the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1, or the region from  $\beta 3$  to  $\beta 4$  having the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1; and

wherein said protein has antithrombotic activity,

and wherein said protein comprises an amino acid sequence of the following (A) or (B):

(A) the amino acid sequence of the amino acid residues 47 to 111 in the amino acid sequence of SEQ ID NO: 1;

(B) the amino acid sequence according to (A), in which the cysteine residue of the amino acid residue 81 in the amino acid sequence of SEQ ID NO: 1 is replaced with an alanine residue.

Claim 26 (Withdrawn): A method for producing a protein having antithrombotic activity, which comprises:

culturing a host microorganism transformed with the DNA as claimed in Claim 25; and collecting the protein from a culture.

Claim 27 (Withdrawn): A DNA coding for a protein comprising an amino acid sequence with a homology of not less than 30% to the amino acid sequence of SEQ ID NO: 1 and forms a secondary or tertiary structure composed of a first  $\beta$  strand ( $\beta 1$ ), a first  $\alpha$  helix ( $\alpha 1$ ), a second  $\alpha$  helix ( $\alpha 2$ ), a second  $\beta$  strand ( $\beta 2$ ), a loop, a third  $\beta$  strand ( $\beta 3$ ), a fourth  $\beta$  strand ( $\beta 4$ ) and a fifth  $\beta$  strand ( $\beta 5$ ) in this order from the amino terminus, and wherein at least one amino acid residue in a region from  $\alpha 2$  to  $\beta 2$ , a region from  $\beta 3$  to  $\beta 4$ , or in the

regions from  $\alpha 2$  to  $\beta 2$  and from  $\beta 3$  to  $\beta 4$  is replaced so that electric charge of the amino acid residue is substituted towards positive direction as compared to the unsubstituted amino acid, said protein being the following (a) or (b):

(a) a protein, in which the region from  $\alpha 2$  to  $\beta 2$  has the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1 and the region from  $\beta 3$  to  $\beta 4$  has the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1;

(b) the protein according to (a), in which substitution, insertion or deletion of one amino acid residue is included in the region from  $\alpha 2$  to  $\beta 2$  having the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1, or the region from  $\beta 3$  to  $\beta 4$  having the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1; and

wherein said protein has antithrombotic activity,

and wherein said protein has the amino acid sequence in which a region containing the loop structure existing between  $\beta 2$  and  $\beta 3$  is deleted in such a manner that the secondary or tertiary structures of  $\beta 2$  and  $\beta 3$  are maintained, or the region is replaced with one or more amino acid residue(s) in a number required to maintain the secondary or tertiary structures of  $\beta 2$  and  $\beta 3$ , said amino acid residue(s) being selected from the group consisting of a glycine residue, an alanine residue, a serine residue and a cysteine residue.

Claim 28 (Withdrawn): A method for producing a protein having antithrombotic activity, which comprises:

culturing a host microorganism transformed with the DNA as claimed in Claim 27;  
and collecting the protein from a culture.

Claim 29 (Withdrawn): The DNA according to Claim 27, wherein the region containing the loop structure existing between  $\beta 2$  and  $\beta 3$  in said protein is replaced with an amino acid sequence composed of four glycine residues.

Claim 30 (Withdrawn): A method for producing a protein having antithrombotic activity, which comprises:

culturing a host microorganism transformed with the DNA as claimed in Claim 27;  
and collecting the protein from a culture.

Claim 31 (Withdrawn; Currently Amended): A DNA coding for a protein comprising an amino acid sequence with a homology of not less than 30% to the amino acid sequence of SEQ ID NO: 1 and forms a secondary or tertiary structure composed of a first  $\beta$  strand ( $\beta 1$ ), a first  $\alpha$  helix ( $\alpha 1$ ), a second  $\alpha$  helix ( $\alpha 2$ ), a second  $\beta$  strand ( $\beta 2$ ), a loop, a third  $\beta$  strand ( $\beta 3$ ), a fourth  $\beta$  strand ( $\beta 4$ ) and a fifth  $\beta$  strand ( $\beta 5$ ) in this order from the amino terminus, and wherein at least one amino acid residue in a region from  $\alpha 2$  to  $\beta 2$ , a region from  $\beta 3$  to  $\beta 4$ , or in the regions from  $\alpha 2$  to  $\beta 2$  and from  $\beta 3$  to  $\beta 4$  is replaced so that electric charge of the amino acid residue is substituted towards positive direction as compared to the unsubstituted amino acid, said protein being the following (a) or (b):

(a) a protein, in which the region from  $\alpha 2$  to  $\beta 2$  has the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1 and the region from  $\beta 3$  to  $\beta 4$  has the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1;

(b) the protein according to (a), in which substitution, insertion or deletion of one or amino acid residue is included in the region from  $\alpha 2$  to  $\beta 2$  having the sequence of the amino acid residues 47 to 72 in the amino acid sequence of SEQ ID NO: 1, the region from  $\beta 3$  to  $\beta 4$

having the sequence of the amino acid residues 94 to 111 in the amino acid sequence of SEQ ID NO: 1 and

wherein said protein has antithrombotic activity,  
and wherein at least one acidic amino acid residue of which  $\alpha$  carbon atom exists within 10 Å from the  $\alpha$  carbon atom of the arginine residue of the amino acid ~~number~~ residue 103 in the amino acid sequence of SEQ ID NO: 1 is replaced with a neutral amino acid residue.

Claim 32 (Withdrawn): A method for producing a protein having antithrombotic activity, which comprises:

culturing a host microorganism transformed with the DNA as claimed in Claim 31;  
and collecting the protein from a culture.

Claim 33 (Withdrawn): The DNA according to Claim 31, wherein in said protein-the acidic amino acid residue to be replaced is composed of at least one residue selected from the aspartic acid residue of the amino acid residue 54, the aspartic acid of the amino acid residue 101 and the glutamic acid residue of the amino acid residue 106 in the amino acid sequence of SEQ ID NO: 1.

Claim 34 (Withdrawn): A method for producing a protein having antithrombotic activity, which comprises:

culturing a host microorganism transformed with the DNA as claimed in Claim 33;  
and collecting the protein from a culture.



SUPPORT FOR THE AMENDMENTS

Claims 12-20 and 24 were previously canceled.

Claims 1, 4-6, 9, 10, and 31 have been amended.

The amendment of Claims 1, 4-6, 9, 10, and 31 is supported by Claims 1-24 as originally filed, as well as the specification as filed at pages 7-85.

The specification has been amended to replace the phrase “amino acid number” with “amino acid residue.” Support for this amendment is found throughout the specification.

No new matter is believed to have been entered by the present amendments.